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Intracellular and extracellular sulphydryl levels in rheumatoid arthritis

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SUMMARY We detected no difference in the reduced glutathione content of erythrocytes obtained from patients with rheumatoid arthritis (RA) and controls. The stability of glutathione to oxidative stress (cumene hydroperoxide) was also the same. Although measured in the erythrocyte, our results indicate that changes in intracellular reduced glutathione are not involved in the aetiology of RA. Serum from patients with RA had a significantly reduced ($p<0.01$) sulphydryl (SH) concentration (415±89 (SD) μmol/l) compared with controls (583±74 μmol/l). This was also valid if the SH groups were expressed per gram of protein. Serum and synovial fluid from RA patients contained similar levels of SH groups (μmol/g protein).

Key words: glutathione, erythrocyte membrane, oxygen free radicals, oxidative stress, cumene hydroperoxide, antioxidants.

It has been reported that the number of free SH groups in the sera of rheumatoid arthritis (RA) patients is depressed.[1 2] This depression is associated with the activity of the disease.[2] Hall *et al.*[3] have shown in vitro that H_2O_2 formed during phagocytosis by granulocytes diminishes the amount of free SH groups. These authors propose that serum SH groups act as important extracellular scavengers of peroxides and are therefore helpful in protecting the surrounding tissues. However it is unlikely that H_2O_2 is formed in the intravascular compartment, whereas H_2O_2 would be expected in the synovial fluid of RA patients due to the activity of phagocytosing cells. It is possible therefore that the concentration of sulphydryl groups/g protein might be lower in synovial fluid than in serum. In this report we compared the concentration of SH groups in serum and synovial fluid of RA patients.

An important, non-protein, free SH is glutathione (GSH). This tripeptide is located almost entirely intracellularly and the negligible amount present in serum can be ignored for the purposes of this study. GSH is very important for normal cell functions. In the context of this report GSH has an important role in the defence against oxidative stress. For example, glutathione peroxidase is involved in the destruction of free peroxidised fatty acids and H_2O_2.[4] Furthermore, glutathione is the cosubstrate for the peroxidation inhibiting protein which is capable of destroying hydroperoxide fatty acids located in phospholipids.[5] Glutathione peroxidase does not possess this latter activity.[4] Oxidised glutathione (GSSG) is reduced by the NADPH (reduced nicotinamide adenine dinucleotide phosphate) dependent enzyme glutathione reductase. NADPH, formed by the pentose phosphate shunt, is important, as it is not only the total amount of GSH which is important for the cell but also the capacity of the cell to reduce the formed GSSG.

It has been reported[6] that the amount of GSH in erythrocytes of RA patients increases after treatment with penicillamine.

It has also been claimed[7] that GSH decreases during relapses and increases during remissions. Since the amount of GSH of RA patients and controls does not differ very much, we have investigated the capacity of erythrocytes from RA patients to reduce GSSG.

Materials and methods

Serum, synovial fluid, and erythrocytes were obtained from patients with classic or definite RA as

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Table 1 *The level of SH groups in the serum and synovial fluid of RA patients (n=5) and in the serum of controls (n=5)*

	SH (μmol/l)	*Protein (mg/ml)*	*SH/g protein (μmol/g protein)*
Control serum	583±74	70·6±2·2	8·26±1·09
RA patient serum	415±89*	70·8±6·7	5·85±1·06*
RA patient synovial fluid	257±64	41·4±6·9	6·23±1·24†

The values are mean±SD.
*$p<0.01$ compared with control.
†Not significant compared with RA serum.

defined by the American Rheumatism Association criteria.[8] Controls and patients were age matched and all below the age of 55.

Protein determinations were performed according to Lowry *et al.*[9] SH groups were assayed with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) as follows: 50 μl serum, 750 μl 0·1 M phosphate buffer (pH 7·4), and 200 μl DTNB (2 mM) were incubated at 37°C for 5 min. The absorbance at 412 nm was measured and a molar extinction coefficient of 13 600 was used. GSH was determined according to Beutler *et al.*[10] The capacity of cells to reduce oxidised glutathione (the so called glutathione stability) was determined according to Koster *et al.*[11]

Results

SERUM AND SYNOVIAL FLUID SH GROUPS

Table 1 shows the amount of SH groups present in the serum and synovial fluid of RA patients and in the serum of controls. The absolute concentration of SH in serum from RA patients was significantly lower than that of healthy controls ($p<0.01$), when expressed as either μmol/l or μmol/g protein. Synovial fluid samples were obtained from the same RA patients. The synovial fluid SH concentration was lower than in serum. No difference was found when the SH concentration was expressed as μmol/g protein.

Table 2 *The GSH level in erythrocytes from RA patients and controls*

	Control (n=4)	*RA (n=6)*
GSH (nmol/ml packed cells)*	1772±144	1898±85

Values are mean±SD.
*nmol/ml=μmol/l.

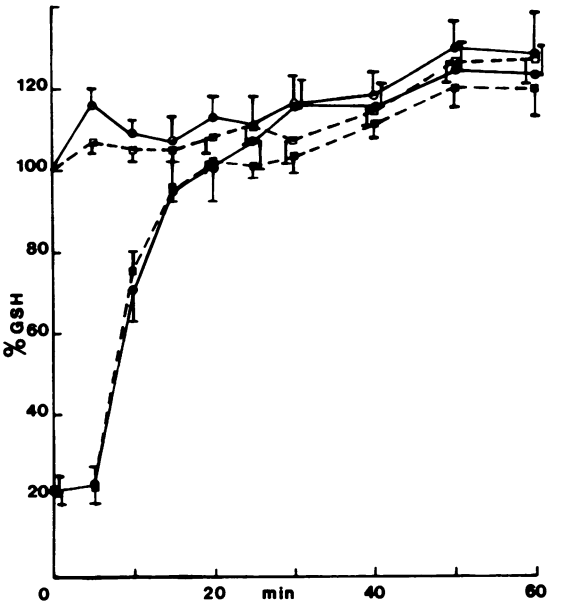

Fig. 1 *The reduced glutathione content of intact erythrocytes from RA patients (n=6) and controls (n=4) during incubation with cumene hydroperoxide (0·5 mM) in the presence of glucose (5 mM). Cumene hydroperoxide was added at zero time, and the values are expressed as a percentage of the initial glutathione content. Controls ○–○ and ●–●; RA patients □–□ and ■–■. Open symbols are in the absence and closed symbols in the presence of cumene hydroperoxide.*

GLUTATHIONE STABILITY IN ERYTHROCYTES FROM RA PATIENTS AND HEALTHY CONTROLS

There was no difference in the amount of GSH (nmol/ml (μmol/l) packed cells) in erythrocytes from RA patients and controls (Table 2).

Fig. 1 compares the ability of erythrocytes obtained from RA patients and healthy controls to regenerate GSH after peroxidative stress. There is the same rapid depletion of GSH upon addition of cumene hydroperoxide and a similar rate of GSH restoration. This indicates that the capacity to maintain adequate GSH levels under peroxidative circumstances is the same for both groups.

Discussion

We have confirmed that sera from RA patients have a reduced amount of SH groups compared with controls. The reduction in serum SH groups is not simply a result of the decrease in serum albumin as shown by Thomas and Evans.[12] Hall *et al.*[3] suggested that the diminished amount of SH groups

may be due to oxidation by H_2O_2 generated by stimulated phagocytes. We were able to confirm these data (unpublished results). In RA patients without extra-articular lesions it is unlikely that circulating granulocytes are stimulated and so would not produce H_2O_2. In the synovial fluid H_2O_2 is to be expected due to phagocytosis. It therefore seemed reasonable to assume that the amount of SH (μmol/g protein) would be lower in the synovial fluid than in the sera of RA patients. However, our data showed that there was no difference in SH groups (per g protein) in these two compartments. It is possible that this is due to a rapid exchange of proteins between the synovial fluid and serum. Another possibility is that the synovial fluid contains a factor(s) which protect(s) the proteins effectively.

Treatment of RA patients with penicillamine leads to an increase in the amount of erythrocyte GSH.[6 7] Although the absolute amount of GSH is important, in order to maintain cellular functions and provide a barrier against peroxidative destruction, the capacity to reduce the formed GSSG is also very important. This reaction enables the cell to withstand stress longer than would otherwise be possible if GSH were not regenerated. We have shown that there is no difference in GSH content of erythrocytes from RA patients and controls. If alterations of GSH levels did have a role in the aetiology of RA, it is possible that the capacity to regenerate GSH during oxidative stress could be reduced. However, erythrocytes from controls and RA patients were equally effective in regenerating GSH when challenged with cumene hydroperoxide. It is therefore unlikely that the increase in erythrocyte GSH, observed on administration of penicillamine, can explain the disease remissions. Furthermore it is not known, at least to our knowledge, what happens to the GSH level in erythrocytes from controls treated with penicillamine.

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